Partitioning of Benzene in Blood: Influence of Hemoglobin Type in Humans and Animals

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Earlier studies have shown that air/blood partition coefficients (PCs) for many volatile organic chemicals (VOCs) are much higher in rat blood than in human blood. It has been suggested that the discrepancy could be attributed to the fact that hemoglobin (Hb) in rat blood exists in a quasi-crystalline form of hydrophobicity greater than that of normal human Hb (HbA) and thus has a higher carrying capacity for VOCs. In the present study, we used benzene as a prototypic VOC to examine its relative partitioning into human and animal blood. Additionally, we sought to ascertain whether the water-insoluble form of hemoglobin (HbS) found in subjects with homozygous sickle cell (SC) disease has a greater VOC-carrying capacity than does HbA blood. At a low-O_2 tension, HbS switches to water-insoluble polymers, which physically deforms the red blood cells (RBCs) to the sickle shape. We equilibrated HbA, HbS, Hartley guinea pig, CD1 mouse, and rat (F-344, Wistar, and Sprague-Dawley) blood and their respective fractions with benzene vapor (80 or 400 ppm) for 3 hr at 37°C in air-tight vials. We introduced benzene vapor into the vial head space that contained air or respiratory mixtures of venous-type (low-O_2) or arterial-type (high-O_2) gases. The blood measurements included the PC, Hb, partial pressures of O_2 (pO_2) and CO_2 (pCO_2), pH, and percentage of SCs. The benzene concentration had no effect on these parameters, and the high- and low-O_2 gas mixtures produced the expected changes in pO_2, pCO_2, and pH. At equilibrium, the low-O_2 HbS blood had approximately 85% SCs compared with roughly 15% with air or high-O_2 gas. PCs for rat and mouse blood were about 100% higher than those for human and guinea pig blood, but the PC for deoxygenated HbS blood was only slightly higher than that for HbA or oxygenated HbS blood. Benzene showed higher affinities for RBCs in the deoxygenated HbS, rat, and mouse blood and higher affinity for plasma in the guinea pig blood. There was no evidence of disproportionate partitioning of benzene into oxygenated HbS or into HbA blood forms. These data suggest that the water solubility of Hb alone appears to have little effect on the VOC-carrying capacity of blood and that the influence of species is large in comparison. These latter differences in partitioning may depend on the number of hydrophobic sites on the surface of the plasma/heme proteins and thus be unique to the species. *Key words:* blood, blood fractions, blood gas, *in vitro* exposure, partition coefficient. *Environ Health Perspect* 110:255–261 (2002). [Online 6 February 2002]
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Volatile organic chemicals (VOCs) are common contaminants of air in the home, in the workplace, and around commercial establishments such as gas stations. Many of these vapor-phase pollutants can be absorbed through the respiratory and/or gastrointestinal tract, the skin, and the eye and then transported by blood to critical target organs. The absorption of a VOC into blood, expressed as the blood/gas partition coefficient (PC), is a critical variable used in physiologic-based pharmacokinetic (PBPK) models to ascertain systemic and tissue distribution. By incorporating metabolic pathways and rates of metabolism, PBPK models have received considerable interest in predictive toxicology and risk assessment (*1*). A study of partitioning profiles for five organic solvents (*n*-hexane, toluene, chloroform, methyl isobutyl ketone, and diethyl ether) in normal human and rat blood indicated that the red blood cell (RBC) is an important carrier for hydrophobic VOCs and that the affinity for the three most hydrophobic VOCs (*n*-hexane, toluene, and chloroform) is substantially greater for rat RBCs than for human RBCs (*2*). Gargas et al. (*3*) measured PCs for 36 VOCs in human and in rat blood and showed that they were greater in rat blood than in human blood for 32 of the compounds. For example, the blood/air PC for benzene was 17.8 for the rat compared with 8.9 for the human. The study by Lam et al. (*2*) showed that many solvents associate with the proteins of hemoglobin (Hb), which have hydrophobic regions (*4*). They speculated that the species differences in VOC solubility may be attributed to differences in the water solubility of Hb.

Rat Hb exists in a quasi-crystalline form containing little water, whereas normal human hemoglobin (HbA) is water soluble. This difference in surface hydrophobicity of Hb may underlie the abilities of the more hydrophobic forms of Hb (such as that found in rat blood) to carry more of a given VOC than does human Hb. Among the human population are persons who are homozygous for the sickle cell disease (SCD) gene and thus have sickle cell hemoglobin (HbS) that—unlike HbA, which stays in solution as the RBCs circulate throughout the body—forms water-insoluble polymers when the O_2 tension drops to levels commonly found in venous blood (~40 mm Hg). The polymerization of deoxygenated HbS causes RBCs to deform into a sickle shape that impedes capillary flow. This abnormal shape also shortens the RBC life span, which can cause anemia in these individuals (*5,6*). In addition, the intracellular polymerization of HbS increases in its surface hydrophobicity (*7*) and may increase VOC affinity in sickle cells (SCs). Furthermore, Farnell and McMeekin (*8*) reported that several substances structurally related to toluene, including benzene, bind with Hb and promote crystallization in concentrated phosphate buffer solutions and that the water solubility of HbS was less than that of HbA and other heterozygous forms of hemoglobin, such as sickle cell trait Hb (HbAS). Examination of the benzene affinity for HbS in both the water-soluble and water-insoluble states is of particular interest because benzene is prototypic in the nature of its physical chemical properties of many VOCs. If the PC of a VOC is related to the water solubility of Hb, then sickled blood may have greater VOC-carrying capacity than does HbA or unsickled HbS blood.

In the present study, we examined the influence of the form of Hb (human, normal or sickle, and animal) on the partitioning of inhaled VOCs into blood. We used benzene, a common air pollutant known to produce

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neurotoxic symptoms as well as hematologic abnormalities and malignancies (*9–14*), as a prototypic VOC to ascertain whether the less water-soluble forms of Hb have greater VOC-carrying capacity than do more soluble forms. We equilibrated samples of blood and plasma from humans and several laboratory rodents (HbA and HbS subjects; Fischer-344, Wistar, and Sprague-Dawley rats; CD1 mice; and Hartley guinea pigs) in air-tight vials with benzene in air or with benzene in arterial-like or venous-like gas mixtures at concentrations of 80 and 400 ppm, similar to those of Midzenski et al. (*13*), who reported medical surveillance results for 15 workers acutely exposed to benzene levels ranging between 60 and 600 ppm for several days while working in the fuel tanks of a ship. In the present study, we assayed blood and plasma samples for benzene PC, pH, pO_2 , pCO_2, Hb, reduced hemoglobin (RedHb), plasma free Hb, and SCs.

Methods

Human samples. We collected blood from 60 informed and consenting adult volunteer subjects (≥ 18 years of age) at the Sickle Cell Clinic of Duke University Medical Center (Durham, NC). Approximately one-half of the blood samples were from SCD patients with > 95% HbS in the blood; the remainder were normal blood samples provided by patients' family members who tested negative for HbS blood; two of these subjects, however, were later found to have the SC trait with HbAS blood, and we excluded their data from the study. We matched SC patients as closely as possible with the normal subjects by age, sex, and race. Table 1 shows subject information. We obtained blood (20–25 mL) by venipuncture, distributed it among four and five standard clinical heparinized tubes, stored it under refrigeration, and analyzed the samples within 24 hr of collection. We tested various fractions of blood. One was unaltered blood, which we refer to as "original" blood. Another was plasma (obtained from blood that was centrifuged at 1,500 rpm and 4°C for 15 min). Other samples were recombinations of plasma and cells (mixtures with high or low RBC content). The latter we refer to as "mixed" blood. In addition, we determined PCs for stroma from human erythrocytes and reconstituted dilutions of fibrinogen and globulin from human plasma (Sigma-Aldrich, St. Louis, MO) in water or saline at varying concentrations to determine benzene PCs.

Table 1. Information regarding adult subjects used in the study.

Characteristics	Normal (HbA)	SCD (HbS)	Trait (HbAS)
Number	28	30	2
Male	8	19	1
Female	20	11	1
Employed	28	17	2
Hb (g/dL)	13.9 ± 0.3	9.0 ± 0.3	12.2
Blood transfusion within 6 months	0	3	0

Animal samples. We used blood from adult male Hartley guinea pigs, CD1 mice, and Fischer-344, Wistar, and Sprague-Dawley rats (all purchased from Charles River Breeding Laboratories, Raleigh, NC) for the study, collected by cardiac puncture under anesthesia with pentobarbital sodium (40 mg/kg intraperitoneal). We processed the animal samples under the same conditions as those used for the human samples. We used blood from seven individual guinea pigs (~600 g body weight) and used pooled blood samples for mice and rats because we needed 1 mL of blood for each equilibration vial, and a large number of vials for the various combinations of blood/gas exposures. Blood from 10 mice (~30 g body weight) provided one pooled mouse sample, and we used nine pooled samples of mouse blood (90 mice). Blood from two rats (~350 g body weight) of like strain provided one pooled sample of rat blood. We used seven pooled samples of rat blood from 14 F-344 rats and six pooled samples each from 12 Wistar and 12 Sprague-Dawley rats, making a total of 38 rats used for the study.

Equipment and procedures. We determined the PC for benzene by measuring the head space concentration of benzene in the test vial and comparing it to the concentration in prepared reference vials, a modified version of the vial-equilibration technique reported by Sato and Nakajima (*15*) and Gargas et al. (*3*). We used head space glass vials with an internal volume of about 12 mL, sealed (air tight) with aluminum crimp caps and Teflon-faced black butyl rubber septa (Hewlett Packard, Wilmington, DE). We agitated the vials and incubated them at 37°C in a vortex evaporator (Haake/Buchler Instrument Inc., Kansas City, MO) fitted with a 20-hole sample block. Because the vial diameter was smaller than the hole in the head block, we used custom aluminum adapters to prevent the vials from breaking.

On an experimental day, we exposed and analyzed blood from two to four subjects or animals. We ran sample vials in duplicate for each of the atmosphere–benzene combinations. Because mixed blood with the high cell count was difficult to pipette accurately, we determined the volumes of the samples indirectly. To do this, we pipetted 1 mL of blood into a preweighed vial and reweighed it to obtain a weight. We calculated the volume using the relative density of blood, adjusted by Hb concentration. At 37°C, HbA blood with Hb equal to 15.9 g/dL has a relative density of 1.05, and that of the normal human blood plasma is 1.02 (*16*). The assumption was that at like concentrations of Hb, the relative density of blood and plasma were similar among the human and the animal types used in this study.

For sample exposure, we placed open vials with blood inside an Atmos Bag (Sigma Diagnostics, St. Louis, MO) for 15 min to equilibrate with air or with a high-O_2 or low-O_2 gas mixture made up to simulate *in*

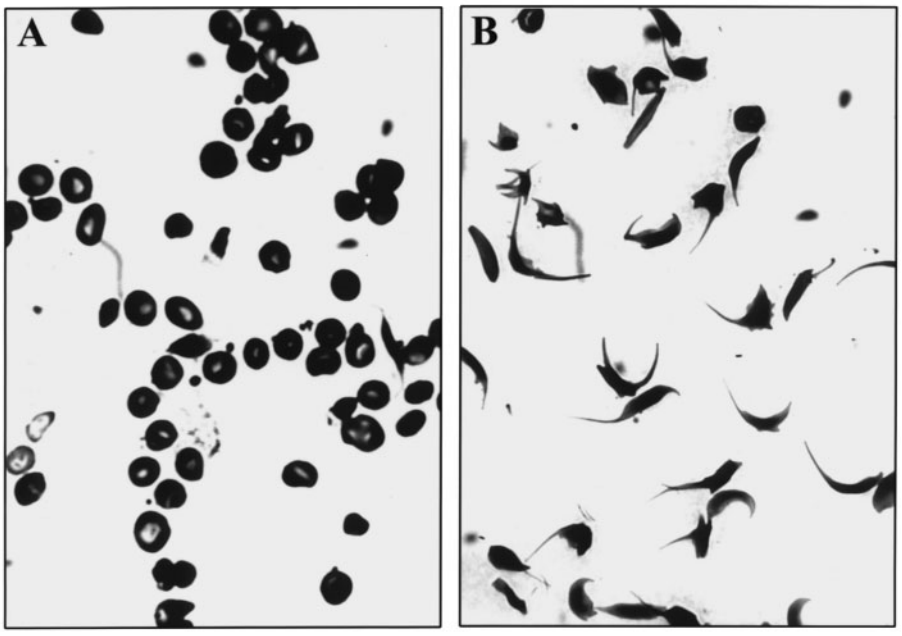

Figure 1. Micrographs of HbS blood smears (magnification × 1,000) that were used for determining the SC count: equilibrated with 400 ppm in a high-O_2 arterial-type (*A*) or a low-O_2 venous-like (*B*) atmosphere.

vivo arterial (16% O_2, 4% CO_2, and balance N_2) or venous (3.5% O_2, 8.5% CO_2, and balance N_2) blood gas tensions. We purchased the gas mixtures from National Welders (Raleigh, NC) and capped the vials while they were still inside the Atmos Bag. The low-O_2 gas tension was adequate to cause the formation of SCs, and the high-O_2 tension, adequate to cause the reversal of the sickling process (*5*). We gently agitated the vials containing blood and six reference vials (containing one drop of distilled water to adjust for water vapor) in the vortex evaporator for 15 min. We made up benzene vapor in Tedlar sample bags (SKC, Eighty-Four, PA) at two concentrations (5,000 and 25,000 ppm). After the 15-min incubation period, we injected 200 µL of benzene vapor or air into the head space of the vial using a gas-tight constant rate syringe (Fisher Scientific, Norcross, GA). This provided a benzene exposure level of 0.0, 80, or 400 ppm for the liquid samples. After an additional 3 hr of incubation and agitation, we then removed 200 µL samples from the head space and injected them into the gas chromatograph for benzene analysis (Hewlett Packard, Avondale, PA). We periodically checked standard curves prepared for benzene.

Immediately after the removal of the gas sample from the head space, we withdrew a blood sample anaerobically with a syringe for the analyses of blood gas, pH, and Hb. The assays of pO_2, pCO_2, and pH used the System 1306 pH/Blood gas analyzer (Instrumentation Laboratories, Lexington, MA). We measured Hb and RedHb on a CO-Oximeter Model 282 (Instrumentation Laboratories, Lexington, MA). For the SC count, we withdrew blood anaerobically from the vial with a disposable 1-mL syringe and 1.5-in, 26-gauge needle and injected one drop of the blood beneath the surface of 1 mL of 10% buffered formalin phosphate and then gently mixed. Later, we made a thin blood smear from the fixed RBC suspension for the SC count. We stained the slides with Diff-Quick (American Scientific Products, McGaw Park, IL) and counted 500 RBCs by microscope with oil immersion. We identified cells as sickled if they were not round or had irregular projected points (Figure 1). This methodology provided a simple and reproducible procedure for the measurement of the percentage of SCs in blood.

We estimated RBC membrane fragility using the colorimetric determination of plasma Hb with a Plasma Hemoglobin Kit (Sigma Diagnostics) and spectrophotometer

Table 2. Human blood and plasma: measurements of blood gases, pH, and RedHb after equilibration with benzene.

Sample type, gas mixture	Benzene concentration (ppm)	pO_2 (mm Hg)	pCO_2 (mm Hg)	pH	RedHb (%)
HbA blood (n = 28)					
Air	400	132 ± 2	17 ± 0.9	7.53 ± 0.06	2.4 ± 0.6
Arterial	0.0	116 ± 1	39 ± 0.4	7.25 ± 0.01	1.5 ± 0.07
Arterial	80	115 ± 1	40 ± 0.5	7.24 ± 1.01	1.6 ± 0.07
Arterial	400	112 ± 1	38 ± 0.4	7.24 ± 0.01	1.7 ± 0.07
Venous	0.0	30 ± 1	55 ± 1.1	7.20 ± 0.01	56 ± 2.1
Venous	80	29 ± 1	59 ± 0.9	7.15 ± 0.01	59 ± 1.5
Venous	400	36 ± 1	51 ± 1.1	7.20 ± 0.01	45 ± 2.5
HbS blood (n = 30)					
Air	400	143 ± 3	20 ± 1.3	7.43 ± 0.05	0.3 ± 0.2
Arterial	0.0	107 ± 2	39 ± 0.6	7.22 ± 0.01	1.6 ± 0.2
Arterial	80	112 ± 2	41 ± 0.9	7.19 ± 0.01	1.1 ± 0.1
Arterial	400	108 ± 1	37 ± 0.4	7.21 ± 0.01	2.7 ± 1.5
Venous	0.0	28 ± 1	57 ± 1.1	7.12 ± 0.01	68 ± 1.5
Venous	80	26 ± 1	62 ± 0.9	7.09 ± 0.01	71 ± 2.3
Venous	400	33 ± 1	53 ± 0.9	7.13 ± 0.01	56 ± 1.6
HbA plasma (n = 8)					
Air	400	148 ± 2	10 ± 0.2	7.98 ± 0.01	—
Venous	400	62 ± 2	33 ± 0.4	7.52 ± 0.01	—
HbS plasma (n = 6)					
Air	0.0	145 ± 2	10 ± 0.4	7.94 ± 0.01	—
Air	400	147 ± 2	11 ± 1.4	7.94 ± 1.79	—
Venous	400	56 ± 3	36 ± 0.5	7.37 ± 0.09	—

Values represent combined data for both original and mixed blood samples (mean ± SE).

Table 3. Human original blood: measurements of hemoglobin, percentage sickle cells, and benzene partition coefficients following equilibration with arterial- and venous-type gas mixtures.

Sample type	Benzene concentration (ppm)	Hb (g/dL)	SCs (%)	PC
HbA blood (n = 17)				
Arterial	80	13.9 ± 0.2	0	8.35 ± 0.24
Arterial	400	13.9 ± 0.2	0	7.97 ± 0.31
Venous	80	13.7 ± 0.2	0	8.40 ± 0.48
Venous	400	13.7 ± 0.2	0	7.87 ± 0.36
HbS blood (n = 15)				
Arterial	80	8.6 ± 0.3*	13.7 ± 1.8*	7.50 ± 0.18
Arterial	400	8.6 ± 0.3*	15.8 ± 2.0*	8.73 ± 0.30
Venous	80	8.5 ± 0.3*	86.5 ± 2.3*, **	6.92 ± 0.27
Venous	400	8.5 ± 0.3*	82.9 ± 2.4*, **	8.68 ± 0.25

Values are mean ± SE.
*Different from HbA blood, $p < 0.05$. **Different from HbS arterial-type blood, $p < 0.05$.

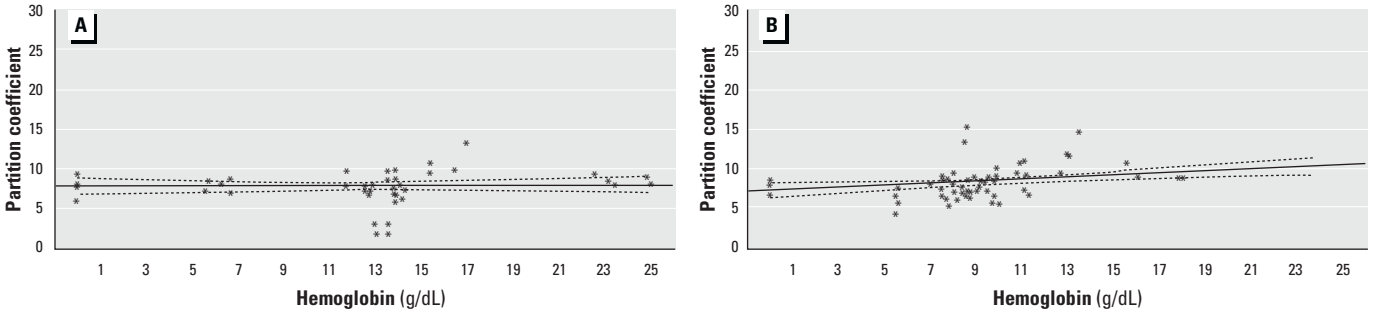

Figure 2. The effect of Hb concentration on partitioning of benzene in deoxygenated HbA (*A*) and HbS (*B*) human blood: slopes from regression analyses of paired PC and Hb values obtained from original blood, plasma, and mixed blood after the 3 hr equilibration at 37°C with 400 ppm benzene in a venous-type atmosphere. (*A*) PC = 7.9 + 0.02 (Hb); $p = 0.63$. (*B*) PC = 7.49 + 0.13; $p = 0.0004$.

(Milton Roy Spectronic 601; Milton Roy Co., Rochester, NY). We stored plasma at –70°C before the Hb analysis.

Statistics. We performed statistical analyses on averaged data (averaged values from duplicate samples). For all responses and interactions between benzene effects and atmosphere effects, we examined variables using a two-way analysis of variance (ANOVA) model. We used linear regression analysis to determine the relationship between the benzene PC and Hb concentration in the blood. For all statistical tests, we selected $p < 0.05$ as the level of significance.

Table 4. Human and animal original blood and plasma: measurements for hemoglobin and liquid/air PCs after equilibration with 400 ppm benzene.

Sample type	Source	Hb (g/dL)	PC
Blood			
Human HbA	6 Subjects	13.4 ± 0.3	8.12 ± 0.4
Guinea pig	7 Guinea pigs	12.4 ± 0.3	8.37 ± 0.3
CD1 mouse	6 Pooled samples	13.6 ± 0.2	17.44 ± 0.7
F-344 rat	6 Pooled samples	13.5 ± 0.7	13.73 ± 0.9
Wistar rat	4 Pooled samples	14.3 ± 0.8	15.05 ± 0.8
Sprague-Dawley rat	4 Pooled samples	13.7 ± 0.2	15.64 ± 0.7
Plasma			
Human HbA	8 Subjects	0.0	8.39 ± 0.4
Guinea pig	7 Guinea pigs	0.0	8.58 ± 0.3
CD1 mouse	6 Pooled samples	0.0	6.23 ± 0.2
F-344 rat	6 Pooled samples	0.0	7.96 ± 1.3
Wistar rat	5 Pooled samples	0.0	6.98 ± 0.8
Sprague-Dawley rat	6 Pooled samples	0.0	9.97 ± 2.0

Values are mean ± SE.

Results

In vitro *exposure.* Measurements of PO_2, pCO_2, pH, and RedHb were similar between HbA and HbS blood and the HbA and HbS plasma following equilibration with the same gas–benzene mixture. For example, blood exposed to the arterial-type gas mixture with 400 ppm benzene had pO_2 values of 112 ± 1 and 108 ± 1 mm Hg for HbA and HbS blood, respectively, and the pCO_2 values were 38.04 ± 0.4 and 37 ± 0.04 mm Hg (Table 2). This agreement of data was also seen in the blood and plasma measurements of the animals (data not shown). Neither the experimental procedure nor the exposure conditions caused hemolysis of red cells in the various exposure groups (data not shown).

Benzene partitioning in human blood. In original samples of HbA and HbS blood, the

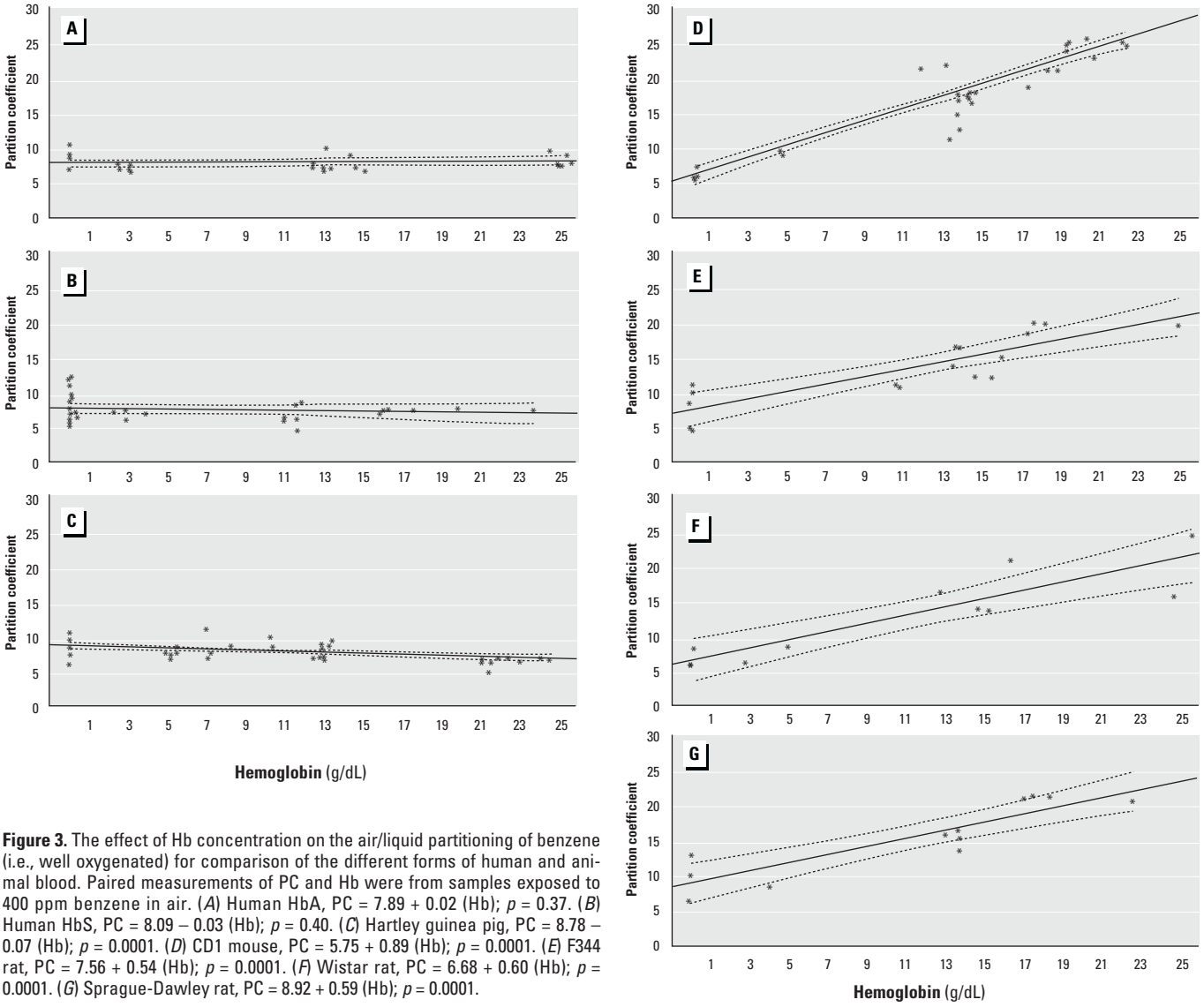

Figure 3. The effect of Hb concentration on the air/liquid partitioning of benzene (i.e., well oxygenated) for comparison of the different forms of human and animal blood. Paired measurements of PC and Hb were from samples exposed to 400 ppm benzene in air. (*A*) Human HbA, PC = 7.89 + 0.02 (Hb); $p = 0.37$. (*B*) Human HbS, PC = 8.09 – 0.03 (Hb); $p = 0.40$. (*C*) Hartley guinea pig, PC = 8.78 – 0.07 (Hb); $p = 0.0001$. (*D*) CD1 mouse, PC = 5.75 + 0.89 (Hb); $p = 0.0001$. (*E*) F344 rat, PC = 7.56 + 0.54 (Hb); $p = 0.0001$. (*F*) Wistar rat, PC = 6.68 + 0.60 (Hb); $p = 0.0001$. (*G*) Sprague-Dawley rat, PC = 8.92 + 0.59 (Hb); $p = 0.0001$.

PCs were not affected by the benzene concentration or by the level of O_2 in the head space; that is, no significant differences were found in PC among the various gas–benzene exposure combinations (Table 3). This uniformity in PC was seen even though there were marked disparities in Hb content between the two Hb variants. Hb content averaged 13.8 g/dL for HbA blood and 8.55 g/dL for the HbS blood. In addition, we saw large differences in the number of SCs among the groups. Venous-type HbS blood showed around 85% SCs, whereas we found only about 15% in the arterial-type and air-exposed HbS blood, and no SCs in HbA blood. The SCs found in HbS blood after the 3-hr exposure to high-oxygen gas indicated an average of approximately 15% irreversible SCs (5) among the SCD subjects.

Because Hb levels were much lower in original HbS blood than in original HbA blood, we examined the effect of Hb concentration on PC. We performed linear regression analyses using paired measurements of Hb and PC. These calculations included the values obtained from original blood, mixed blood, and plasma assays. Hb concentration among these samples ranged between 0 and roughly 26 g/dL, and all of the samples had been exposed to 400 ppm benzene. Figure 2 illustrates the relationship between sickled cells and PC by plotting Hb versus PC for venous-type HbA blood with no SCs (Figure 2A) and venous-type HbS blood with approximately 85% sickled cells (Figure 2B). The regression for HbA blood is PC = 7.90 + 0.02 (Hb) with a slope not different from zero (p = 0.63), whereas the slope for HbS blood is PC = 7.49 + 0.13 (Hb) and is significantly positive (p = 0.0004). These analyses indicate that benzene partitioning in blood with no SCs is similar for RBCs and plasma, but when there are a large number of SCs, the partitioning is somewhat greater in RBCs.

Liquid/Air PCs for benzene in the blood of normal humans and animals. Table 4 shows original-blood/air and plasma/air PC values after equilibration with 400 ppm benzene. We present the air data because previous studies have used air as the head space gas, and these data allow for a direct comparison with the data already published. These measurements show that benzene partitioning was not significantly different between HbA blood and guinea pig blood (8.12 vs. 8.37) at comparable Hb levels. This similarity was also found for HbA plasma and guinea pig plasma (8.39 vs. 8.58). In contrast, the PCs for mouse and rat blood samples were much higher at comparable Hb levels, with the mouse being 17.44 and the rats being 13.73, 15.05, and 15.64 for F-344, Wistar, and Sprague-Dawley strains, respectively. These high PCs were not seen in the plasma, suggesting that there is more benzene associated with the RBCs in these rodents.

Figure 3 plots the influence of Hb content on blood/air PC for these groups using regression analysis. The slope in Figure 3A for human HbA blood, PC = 7.89 + 0.02 (Hb), is not different from zero (p = 0.37) and almost identical to the slope for venous-type HbA blood shown in Figure 2A. The regression equation for well oxygenated HbS blood (Figure 3B), with only about 15% sickled cells, is PC = 8.09 + 0.03 (Hb), and like the blood/air slope for HbA blood, the slope was not different from zero (p = 0.40). It was, however, different from the slope derived from the highly sickled venous-type HbS blood shown in Figure 2B.

The regression for guinea pig blood, PC = 8.78 ± 0.07 (Hb) in Figure 3C, showed a slightly negative slope (p = 0.0001) and suggests that benzene may be a little less soluble in RBCs than in plasma. On the other hand, regressions for the mouse and the three strains of rats (Figure 3D–G) all had strong and significantly positive slopes, with PCs = 5.75 + 0.89 (Hb) for the mouse, and 7.56 + 0.54 (Hb), 6.68 + 0.60 (Hb), and 8.92 + 0.59 (Hb) for F-344, Wistar, and Sprague-Dawley rats, respectively, indicating that benzene solubility is decidedly greater in the RBCs than in plasma for these rodents.

To compare PCs among human and animal blood, we offset the effect of Hb concentration by normalizing PC for Hb concentration and selected 14 g/dL of Hb arbitrarily for these calculations. The normalized values for PC (Table 5) indicate that well-oxygenated HbA, deoxygenated HbA, and well-oxygenated HbS blood had comparable PC values (8.51, 8.18, and 8.17, respectively), but the PC for deoxygenated HbS blood was somewhat higher (9.31). Comparisons among blood/air PCs for human (HbA) and animal blood again indicated that the PC for guinea pig blood is similar to that found for humans (7.80 vs. 8.51), and both are approximately 50% less than those found for the mouse (18.21) and rat (~16) blood.

Benzene liquid/air PCs for blood fractions. Benzene did not appear to be particularly soluble in some of the other components derived from normal HbA blood (stroma, fibrinogen, or globulin). The benzene water/air and saline/air PCs did not show significant changes when these components were added over a wide range of concentrations (Table 6). We saw a small increase in PC with stroma as the concentration reached 20 mg/mL. However, a linear regression of the data indicated that the correlation fell short of significance ($p < 0.05$), and a graph of the data did not strongly suggest a linear relationship among the variables.

Table 5. Human and animal blood: calculated benzene blood/air PC, normalized to 14 g/dL of hemoglobin.

Type of blood	Equilibration atmosphere	SCs (%)	PC
Human HbA	Air	0	8.51
Human HbA	Venous gas mixture	0	8.18
Human HbS	Air	~15	8.17
Human HbS	Venous gas mixture	~85	9.31
Hartley guinea pig	Air	0	7.8
CD1 mouse	Air	0	18.21
Fischer-344 rat	Air	0	15.12
Wistar rat	Air	0	15.08
Sprague-Dawley rat	Air	0	17.18

Samples were equilibrated with 400 ppm benzene in air.

Table 6. Deionized water, saline, and blood fractions: measurements of benzene liquid/air PCs after equilibration with 400 ppm benzene.

Sample type	No. samples in liquid	PC
Deionized water	3	2.77 ± 0.02
Normal saline	6	2.23 ± 0.03
HbA RBC stroma (Sigma)		
1 mg/mL	6 Deionized water	2.30 ± 0.3
5 mg/mL	2 Deionized water	2.12
10 mg/mL	2 Deionized water	2.69
20 mg/mL	2 Deionized water	3.23
HbA fibrinogen (Sigma)		
1 mg/mL	2 Normal saline	2.45
5 mg/mL	2 Normal saline	2.93
10 mg/mL	2 Normal saline	2.12
HbA globulin (Sigma)		
10 mg/mL	2 Deionized water	2.38
25 mg/mL	2 Deionized water	1.77
50 mg/mL	2 Deionized water	2.65

Values are mean ± SE.

Discussion

In this study we used benzene as a prototype to examine the partitioning of VOC into blood with various forms of Hb. Earlier studies have shown that the blood/air partitioning for many VOCs is much higher in rat blood than in human (HbA) blood (*2,3*) and that the more hydrophobic solvents partition disproportionately into RBCs (*2*). The difference in Hb affinity for VOCs between human and rat blood has been attributed to the observation that the rat Hb exists in a quasi-crystalline form inside the RBC, implying that it is more hydrophobic than human Hb, which is water soluble (*2*). Our results suggest that the extent of VOC partitioning in blood may be much more associated with species differences than with the water solubility of Hb per se and that the disproportionate partitioning of benzene into RBCs may be species dependent.

Our benzene blood/air PC value for HbA blood (8.12 ± 0.4; Table 4) agreed with the blood/air PCs determined by Sato and Nakajima (*15*) and Gargas et al. (*3*), who reported 7.8 and 8.19 ± 0.10 (SE), respectively. All of these human PC values were higher than that reported by Fiserova-Bergerova et al. (*17*), which was 6.4 ± 0.6 (SE). However, Fiserova-Bergerova et al. used a short 30-min equilibration period that may have underestimated the PC value. Like Gargas et al. (*3*), we found that the benzene PC for rat blood was much higher than that for original human blood, although their actual values for the F-344 rat and the CD1 mouse were not in agreement with ours. Their rat PC was 17.8 ± 0.3 (SE) compared with our 13.7 ± 0.9, and their mouse PC was 12.1 ± 0.3 (SE) compared with our 17.4 ± 0.7. Interestingly, the other two rat strains (Wistar and Sprague-Dawley) had PCs of 15.05 ± 0.8 and 15.64 ± 0.7, respectively, similar to those for F-344 rat blood. Surprisingly, the PC for guinea pig blood (8.37 ± 0.3) was more like that found for human blood than for other rodent blood. These data, shown in Table 4, indicate a strong species effect on benzene partitioning in blood.

We investigated the influence of water solubility of Hb on benzene partitioning using human HbA and HbS blood because the HbS variant becomes water insoluble and forms hydrophobic polymers when deoxygenated (*7,8*). We reasoned that if VOC partitioning in blood is related to the water solubility of Hb, then a switch in the water solubility of HbS should also cause a switch in the PC. We addressed this hypothesis by measuring benzene PCs following equilibration with high-and low-oxygen gas mixtures. After the 3-hr equilibration, the well-oxygenated samples of blood (arterial-type and air) had PO_2 levels between 105 and 145 mm Hg, with less than 3% RedHb. The venous-type blood had PO_2 levels of about 30 mm Hg, with around 60% RedHb. The oxygenated samples of HbS blood had about 15% irreversible SCs, compared with about 85% SCs in the venous-type samples (no RBC deformities were found in any other group). The PC values for HbA and HbS original blood were not different among the oxygenated and deoxygenated forms (Table 3), suggesting that there was no effect of the water solubility of Hb on the affinity of benzene. However, the Hb concentration in HbS blood (~8.6 mg/dL) was significantly lower than in HbA blood (~13.9 g/dL). Therefore, it was possible that benzene did partition disproportionately into sickled RBCs but was not detectable because of the discrepancy in Hb concentrations.

We examined the effect of Hb concentration on benzene PC using regression analysis for PC versus Hb (between 0.0 and 25 g/dL). The HbA blood data showed no slope for any gas–benzene combination (Figures 2A and 3A), indicating that benzene was equally partitioned between plasma and RBCs over a wide range of plasma–Hb combinations. For the deoxygenated HbS blood (Figure 2B), there was a small but significant positive slope. However, no slope was seen for the oxygenated HbS blood form (Figure 3B). These results demonstrate that when HbS switched from the soluble form to the insoluble form, there was a slight increase in the partitioning of benzene into RBCs. When we normalized benzene PCs to 14 g/dL Hb (Table 5), the recalculated PC for deoxygenated HbS blood (9.31) was higher than that of the oxygenated HbS blood (8.17) as well as the oxygenated and deoxygenated HbA blood (8.51 and 8.18, respectively), indicating that there was an effect of the water solubility of Hb on the PC, albeit small (~15%).

The PC versus Hb regression analyses for the three rat strains and the mouse produced marked positive slopes for all benzene–gas combinations (Figure 3D–G), indicating that benzene had more affinity for RBCs than for plasma and that partitioning was not affected by the O_2 partial pressure. The PC slope for guinea pig blood, however (Figure 3C), was slightly but significantly negative, suggesting that benzene may partition a little more in plasma than in RBCs. When we adjusted the PCs for these blood forms for an Hb concentration of 14 g/dL, the calculated PCs for rat blood were similar among the three strains, 15.2, 15.08, and 17.18 for F-344, Wistar, and Sprague-Dawley rats, respectively, and a little less than that found for mouse blood, which was 18.21. These adjusted PCs were about twice as high as those found for the adjusted human and guinea pig PCs (Table 5). Except for HbS blood, the Hb concentrations in original blood samples were similar among the various forms of blood (Table 4), but it is possible that the differences in PC between our data and those reported by Gargas et al. (*3*) for the F-344 rat and CD1 mouse reflect variations in Hb concentration.

Farnell and McMeekin (*8*) reported that benzene and other cyclic organic compounds promote the crystallization of Hb in concentrated salt solutions and that the HbS form was less soluble than HbA and other human heterozygous forms, such as HbAS. We equilibrated HbS blood in the presence of 0.0, 80, and 400 ppm benzene in high- and low-O_2 gas mixtures and found no effect of benzene on the number of SCs, suggesting that at these concentrations benzene did not appear to alter the crystallization processes of HbS within blood cells.

In this study, the water/air and saline/air PCs for benzene were 2.77 ± 0.02 and 2.33 ± 0.03, respectively, similar to the result found by Gargas et al. (*3*), which was 2.75 ± 0.10 (SE) for saline/air. We examined benzene partitioning into human erythrocyte stroma, fibrinogen, and globulin and found that none were different from benzene partitioning into water or saline, and each PC was much less than that for plasma and RBCs (Table 6).

In conclusion, our results suggest that the hydrophobic regions of blood/heme proteins play significant roles in the partitioning of benzene from air into blood. Moreover, the variability in PCs among species may depend on the differences in these species-specific proteins and the form of Hb (water soluble, quasi-crystalline, or polymerized) may not be the dominant feature underlying the partitioning. The relative number of these hydrophobic sites among the plasma and heme proteins may determine the extent of disproportionate partitioning of VOCs into the RBCs. If the ratio is greater or less than one, an adjustment in Hb concentration may be helpful for comparative purposes. Rat and mouse blood (in contrast to human and guinea pig blood) appear to partition benzene disproportionately into Hb, and this difference may have significant implications when applying these data to PBPK estimates of exposure.

On the basis of the idea that the water solubility of Hb may have a strong effect on the VOC-carrying capacity of blood, we had hypothesized that the deoxygenated form of HbS blood might show a dramatic increase in benzene partitioning, which in theory could intensify the health risk factors associated with benzene toxicity for persons with SCD. The fact that there was approximately

12% more benzene in the deoxygenated HbS blood than in the oxygenated HbS blood at an Hb concentration of 14 g/dL may be chemically quite significant. However, as a result of the transient process whereby the HbS reversibly polymerizes and the fact that SCD patients are anemic, SCD blood carries a similar amount of benzene as the blood of a normal person. Therefore, we can only conclude that these people are probably at no undue exposure risk.

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